

REMARKS

Claims 1, 3-5, 7, 8, 10-23 are pending in this application. Claims 10-23 have been withdrawn. Claims 1, 3-5, 7 and 8 stand rejected under §§ 102(b) or 112, second paragraph. Claims 1 and 3-4 are cancelled. Claims 5 and 7-8 have been amended.

In an effort to simplify prosecution and expedite allowance, Applicants have amended claims 5 and 7 to clarify the claims and to overcome the rejections. Support for amendments to claim 5 may be found throughout the specification, for example, paragraphs [0046] and [0047] of the printed U.S. Patent Publication 2005/0250167. Support for claim 7 may be found in paragraphs [0020] and [0034], for example. It is believed that no new matter has been added.

Interview with the Examiner

On January 25, 2010, the Applicant's representative spoke with the Examiner regarding proposed amendments to claims 5, 7 and 8. The Examiner suggested to add the language, "of PKB Ser 473", after the claim language in the fifth line reciting, "the measurable kinase activity," for further clarity. The Applicants' representative also suggested to add "a" instead of "the" in the 3rd line of claim 7, and to add "complex" in the first line of claim 8. After further review, the Applicants' representative makes further changes to claim 5 and to delete the word "kinase protein complex," in order to be consistent with claim 7. In addition, further changes were made to claim 7 and 8, for clarity, without limiting the scope of the claims.

Rejection under Section 102(b)

Brown, Jolly and Cravatt fail to anticipate the claims because they fail to disclose the limitations of the claims. In order to anticipate, a reference must disclose each and every limitation of the claim exactly. With regard to claim 5, the references fail to disclose the limitations reciting, "... PKB Ser 473 kinase activity and an apparent molecular weight of 450-650 kDa when fractionated by gel filtration chromatography, wherein the purified PKB Ser 473 kinase complex is a PKB Ser 473 kinase complex that has been purified from a cell-free extract." With regard to claim 7, the references fail to disclose the limitations of the claim reciting, "...purified cell extract that has measurable PKB Ser 473 kinase activity in 0.2 µg of protein when detected in a kinase assay in which a PKB peptide substrate is phosphorylated with ³²P labelled phosphate, wherein a kinase complex elutes with an apparent molecular weight of 450-650 kDa

when fractionated by gel filtration chromatography and the measurable PKB Ser 473 kinase activity in the purified cell extract is at least 2000 times greater than a PKB Ser 473 kinase activity in a crude extract, wherein the kinase activities are measured using the kinase assay.” Turning to Brown, Brown merely discloses a general preparation of a cell-free extract comprising a Site-1 protease, which has nothing to do with the protein Kinase B pathway. The additional reference of Jolly, simply discloses a membrane-free extract of 293 cells expressing a human peripheral benzodiazepine receptor, which again has no relevance to the compositions, extracts, and complexes disclosed by the Applicant. Cravatt merely discloses a membrane-free extract of 293 cells expressing an acylpeptide hydrolase, and fails for similar reasons. Thus, none of the references supplement Toker and nowhere disclose a purified PKB Ser 473 kinase complex having the limitations recited in amended claim 5 and a purified cell extract having the limitations recited in amended claim 7.

Toker fails to anticipate for several reasons, because subsequent articles showed that PKB/Akt does not autophosphorylate and thus do not show PKB Ser473 activity, thereby showing that the kinase complex disclosed by the Applicants is distinct from PKB/Akt. Although Toker et al. shows that PBK/Akt has PKB Ser 473 kinase activity, the accuracy of this has been questioned by those skilled in the art. As cited in Applicants’ previous response, Hill et al., *J. Biol. Chem.* (2001) 276(28):25643-6 shows that staurosporine, a broad-specificity kinase inhibitor, potently inhibited PDK1 activity without affecting Ser 473 phosphorylation, which suggests that phosphorylation of Ser473 of PKB is via a distinct Ser-473 kinase and not through PDK1 or PKB activity. In fact, Alex Toker himself acknowledges such skepticism in a later article titled “Akt signaling: A Damaging Interaction Makes Good”, *Trends in Biochemical Sciences*, (2008) 33(8):356-359 (“Second Toker Article”)¹ where he recognizes that the “[i]nitial findings that PDK-1, the integrin-linked kinase or Akt itself was the Ser473 kinase [] were met with considerable skepticism” and acknowledges a study by the Sabatini group which shows the relevant Ser 473 kinase. *Id.* at p.358. These two articles, therefore, suggest that PKB/Akt does

¹ It is noted that the Second Toker Reference was cited to support an argument against that raised by the Examiner during the final office action and to provide information about the state of the art after the filing date of the current application and therefore should not be construed to be an admission that the information cited is material to patentability or prior art.

not autophosphorylate. This conclusion (i.e., that PKB/Akt does not autophosphorylate) is also well known/accepted in the art. As such, the Examiner has not established that PBK/Akt has PKB Ser473 kinase activity.

Furthermore, the purified kinase complex is distinct from PKB/Akt as it has a different molecular weight. Even if the PBK/Akt arguably has PKB Ser 473 kinase activity, the prior art cited does not disclose, explicitly or inherently, the molecular weight limitation of the claims. Nothing in the prior art therefore suggests a complex having a molecular weight of 450-650kDa. In fact, prior to the filing of the current application, it is not known that Ser473 kinase operates via a multi-subunit/protein complex as disclosed in the currently claimed invention. This discovery, however, is consistent with the later finding by the Sabatini group as discussed in the Second Toker Article, which shows that the relevant Ser 473 kinase, in fact, operates as complex.

Moreover, the Toker reference teaches away from the claims and cannot disclose the purified kinase complex, either explicitly or inherently. Toker states that Ser-473 phosphorylation requires the activity of Akt (autophosphorylation) and “cannot result from contaminating kinases or from phosphorylation by PDK-1, as suggested recently.” (paragraph 1, pg. 8274.) This precludes the possibility of a kinase complex having an apparent molecular weight of 450-650 kDa, as recited in the claim. Therefore, the claims are not anticipated by Toker and any of the references.

Furthermore, the methods to study the purified kinase complex are different from those disclosed by Toker. Toker *et al.* uses and discloses a pure Akt which has a MW of 62-64 kDa. (See pg. 8273, 2nd paragraph, under Results and Discussion.) Pg. 8273 pointed out by the Examiner in the previous Office Action does not disclose a protein complex that has a MW of 450-650 kDa as claimed by the Applicants. The reference at the cited page, at most, discloses a wild-type and a mutant AKT in complex with anti-HA antibodies. On the other hand, the claimed complex, was isolated using a very specific method. (See paragraphs [0010], [0012]-[0017], and specifically, paragraph [0014]), wherein an attempt to over-purify, e.g., using ion exchange chromatography resulted in loss of the activity of the complex.) It is respectfully submitted that Akt disclosed in Toker isolated simply using anti-HA antibodies is not the protein complex disclosed by the Applicants.

Furthermore, the specification itself identifies numerous reasons for distinguishing the kinase complex disclosed by the applicants from PDK-1 and autophosphorylation. First, location distinguishes the kinase complex from PKB and PDK-1. The kinase complex was substantially enriched in the plasma membrane fraction, unlike PKB and PDK-1, which are mainly located in the cytosolic fraction. For example, paragraph 102 of the published application notes that the total kinase complex was substantially more in the plasma membrane fraction and specific activity for Ser 473 phosphorylation was found to be highly enriched in the plasma membrane fraction of HEK 293 cells. In contrast, PKB and PDK1 are mainly located in the cytosolic fraction of unstimulated cells, as disclosed by the applicant in the same paragraph. Indeed, the Anderson reference, submitted with this response, shows that PDK-1, generally in the cytosol, translocates to the plasma membrane under certain conditions, such as PDGF stimulation. (See pg. 689, first paragraph.) This reaffirms the differences between the kinase complex identified by the Applicants and the PDK-1. Therefore, the claims are not anticipated by any of the references.

The Ser 473 kinase complex is associated with the plasma membrane and is not an integral membrane protein, according to paragraphs in the specification. For example, paragraph 51 discloses that extraction of the plasma membrane fraction with a high ionic strength buffer releases the Ser473 kinase from the lipid bilayer, thus showing that this kinase complex is not an integral membrane protein. Furthermore, paragraph 114 shows that further attempts to purify the Ser 473 kinase complex resulted in a loss of activity, thus affirming that this disclosed kinase is a kinase complex. Thus, the Applicants distinguish PDK-1 from the purified kinase complex disclosed by the applicants.

Also, the purified kinase complex disclosed by the Applicants is distinct from PKB because PKB isoforms could be phosphorylated under conditions where PKB could not. For example, the applicants teach that phosphorylation of Ser 473 does not occur through autophosphorylation but through the disclosed kinase complex. Paragraph 102 discloses that PKB does not autophosphorylate on Ser 473, under conditions when even the partially purified Ser473 kinase preparation could phosphorylate various PKB isoforms, including a kinase inactive mutant of PKB-alpha. In addition, Figure 2 (corresponding to Example 3) show that the PKB Ser 473 kinase activity eluted in fractions 17-20, and phosphorylation of the recombinant PKB

protein was measured using phospho specific-Ser 473 specific antibodies. Furthermore, using a highly purified crystallization grade PKB, no autophosphorylation of PKB could be seen and thus phosphorylation of Ser 473 was dependent upon addition of the partially purified Ser 473 kinase. Furthermore, Example 5 discloses that PKB isoforms and mutants were not phosphorylated on Ser473 when incubated in kinase reaction buffer, thus showing that PKB does not autophosphorylate on Ser 473. Therefore, the kinase complex is distinguished from the references.

The purified kinase complex disclosed by the Applicants can phosphorylate PKB isoforms and is thus distinct from the autophosphorylation mechanism identified by Toker. For example, these PKB isoforms when incubated with even partially purified Ser473 kinase, with the exception of a PKB with a mutated Ser473 site, were all phosphorylated on Ser473. Even the ATP binding site mutant K179A and the activation loop phosphorylation site mutant T308A (PKB mutants) were not impaired in their ability to be phosphorylated on Ser473, thus showing that PKB kinase activity is not required for maximal Ser473 kinase activity. (See paragraph [0116], for example.) Thus, the claims are not anticipated by any of the references.

Indeed, the purified kinase complex disclosed by the Applicants can thus phosphorylate the activation loop mutants where Toker would affirm that phosphorylation could not occur. In fact, Toker teaches an opposite mechanism from those disclosed by the Applicants and states that phosphorylation at the activation loop triggers autophosphorylation of Ser473.(pg. 8273, last paragraph.) However, the activation loop mutant T308 disclosed in the Applicant's examples *maintained phosphorylation*, thus showing that the phosphorylation occurs through the Ser 473 kinase disclosed by the Applicants, and does not operate through autophosphorylation. If the autophosphorylation mechanism identified by Toker was the mechanism, then the activation loop mutant would not have been phosphorylated. Therefore, the applicants' experimental evidence in the specification affirms that the Ser473 kinase is distinct from autophosphorylation identified by Toker and the references.

Applicants respectfully submit that the references do not explicitly or inherently disclose every element of the claimed invention. Withdrawal of the rejections under 35 U.S.C. § 102 is earnestly requested.

Rejection under 35 U.S.C. § 112, Second Paragraph

The Examiner rejected claim 7 under Section 112, second paragraph for failing to point out and distinctly claim the subject matter for which applicant regards as his invention. The kinase activities are measured using the same metric, and thus, the Applicants amend claim 7 to overcome the objections.

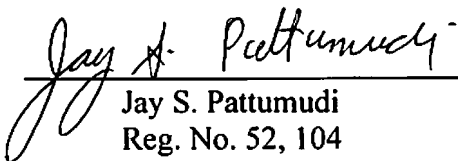
Conclusion

Applicants respectfully submit that the claims are in condition for allowance and notification to that effect is earnestly requested. The Examiner is invited to telephone the below-identified attorney or a representative of the Firm, if the undersigned is not available, to facilitate prosecution of this application. However, please do not leave voicemails at the undersigned's phone number.

It is believed that no fees are due. If any other additional fees are presently required, or required during the pendency of this application (other than payment of the issue fee), the Commissioner is authorized to charge any additional fees, if needed, or credit any overpayment, to deposit account No. 50-4255. If there are any questions, especially ones relating to payments or this response, please contact the undersigned, or if the undersigned is not available, please contact a representative of the firm. Please do not leave voicemails at the undersigned's phone number.

Respectfully submitted,

Date 1/26/10


Jay S. Pattumudi

Reg. No. 52, 104
Hoxie & Associates LLC
75 Main Street, Suite 301
Millburn, NJ 07041
973-912-5232 phone
973-912-5236 fax

JSP